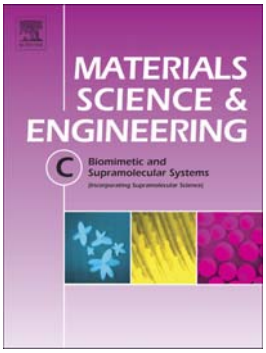
Accepted Manuscript



A novel coping metal material CoCrCu alloy fabricated by selective laser melting with antimicrobial and antibiofilm properties

Ling Ren, Kaveh Memarzadeh, Shuyuan Zhang, Ziqing Sun, Chunguang Yang, Guogang Ren, Robert P. Allaker, Ke Yang

PII: S0928-4931(16)30502-1
DOI: doi: 10.1016/j.msec.2016.05.069
Reference: MSC 6559

To appear in: *Materials Science & Engineering C*

Received date: 26 February 2016
Revised date: 19 April 2016
Accepted date: 16 May 2016

Please cite this article as: Ling Ren, Kaveh Memarzadeh, Shuyuan Zhang, Ziqing Sun, Chunguang Yang, Guogang Ren, Robert P. Allaker, Ke Yang, A novel coping metal material CoCrCu alloy fabricated by selective laser melting with antimicrobial and antibiofilm properties, *Materials Science & Engineering C* (2016), doi: 10.1016/j.msec.2016.05.069

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

A novel coping metal material CoCrCu alloy fabricated by selective laser melting with antimicrobial and antibiofilm properties

Ling Ren[1, a], Kaveh Memarzadeh[1, b], Shuyuan Zhang [a], Ziqing Sun [a], Chunguang Yang [a], GuogangRen[c], Robert P. Allaker[*, b], Ke Yang[*, a]

a. Institute of Metal Research, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang, China 110016

b. Institute of Dentistry, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, Newark Street, London E1 2AT UK

c. University of Hertfordshire, Hatfield AL10 9AB, UK

[1] These authors contributed equally to this work.

[*]: Corresponding authors：Fax:+86 24 23891320, E-mail: kyang@imr.ac.cn; Tel: + 44 20 7882 2388, E-mail: r.p.allaker@qmul.ac.uk

Abstract：

Objective: The aim of this study was to fabricate a novel coping metal CoCrCu alloy using a selective laser melting (SLM) technique with antimicrobial and antibiofilm activities and to investigate its microstructure, mechanical properties, corrosion resistance and biocompatibility.

Methods: Novel CoCrCu alloy was fabricated using SLM from a mixture of commercial CoCr based alloy and elemental Cu powders. SLM CoCr without Cu served as control. Antibacterial activity was analyzed using standard antimicrobial tests, and antibiofilm properties were investigated using confocal laser scanning microscope. Cu distribution and microstructure were determined using scanning electron microscope, optical microscopy and X-ray diffraction. Corrosion resistance was evaluated by potential dynamic polarization and biocompatibility measured using an MTT assay.

Results: SLM CoCrCu alloys were found to be bactericidal and able to inhibit biofilm formation. Other factors such as microstructure, mechanical properties, corrosion resistance and biocompatibility were similar to those of SLM CoCr alloys.

Significance: The addition of appropriate amounts of Cu not only maintains normal beneficial properties of CoCr based alloys, but also provides SLM CoCrCu alloys with excellent antibacterial and antibiofilm capabilities. This material has the potential to be used as a coping metal for dental applications.

ACCEPTED MANUSCRIPT

Key Words: Selective Laser Melting, CoCrCu Alloys; Antimicrobial; Antibiofilm; Coping Metal

1. Introduction:

The Selective Laser Melting (SLM) technique has recently been introduced for fabricating metal coping. In comparison with the traditional casting method, SLM offers several advantages, such as a higher definitive product density, reduced manufacturing time and costs, minimization of human errors, and the prevention of casting defects [1-4]. The SLM technique is also attracting particular interest in prosthetic dentistry. Biofunctionality and biocompatibility are important considerations for the selection of dental alloys [5]. Currently, CoCr based alloys are widely used in prosthodontics due to a combination of favorable properties, including mechanical strength, ductility, corrosion resistance and biocompatibility, which makes them broadly applicable in a variety of applications with fixed and removable restorations. CoCr based alloys, based on SLM technology, were recently introduced for manufacturing base metal coping[6, 7].

It has been suggested that the diversity of bacteria in an individual oral cavity is in the region of 500 species, which can readily colonize the different types of surfaces including teeth, prosthetic devices and dental implants [8-10]. The formation and maturation of biofilms on dental biomaterial surfaces may lead to the development of peri-implant diseases, such as peri-implant mucositis or peri-implantitis, influencing the long-term implant success [11-13]. With respect to such complications, oral biofilms comprise complex three-dimensional structures consisting of diverse multispecies communities as formed on oral tissues and prosthetic devices [14, 15]. Although treatment can provide immediate benefits with respect to elimination of the biofilm or prevention of secondary infection, the prospect of long-term clinical success relies more so on the antimicrobial properties of the dental materials used [16]. Hence, a dental biomaterial that creates a sustained antimicrobial environment around the restoration and is able to discourage biofilm formation would be of considerable clinical benefit [17]. To this end, several antimicrobial agents have been incorporated within restorative materials and bonding systems [16].

Some antimicrobial agents are often immobilized in dental materials used to kill bacteria, such as chlorhexidine (CHX) and 12-metacryloyloxydodecylpyridinium bromide (MDPB) [18, 19]. However, the copper ions is completely unique inorganic bactericide, which is not only an essential metal trace element required to maintain healthy cellular functions in the human body, but also is an alloying element in many alloys systems used in material science. Moreover, copper ions are also well known for its strong antibacterial and antifungal effects as used in human daily life [20, 21]. This provides the impetus to immobilize copper (Cu) into the currently used CoCr

ACCEPTED MANUSCRIPT

alloy, and to develop a new CoCrCu based alloy as manufactured by SLM technology, which could be used to kill microorganisms and inhibit biofilm formation within the oral cavity. Additionally, because of proven toxic effects of Ni, this element has been removed from this new alloy [22, 23].

Thus, the aim of the current study was to evaluate the bactericidal, antibiofilm, corrosion resistance, mechanical, and biocompatibility properties of a novel CoCrCu alloy fabricated by means of SLM technology, in order to provide a preliminary platform for further study and potential clinical applications for an antimicrobial metal coping material.

2. Materials and methods

2.1 Materials

The new CoCrCu alloy was fabricated using SLM from a mixture of commercial CoCr based alloy and elemental Cu powders. Based upon laser particle size analysis (Mastersizer2000, Malvern, UK), the average powder size for CoCr alloy and elemental Cu were 25 and 45 μm, respectively. The fully dense CoCrCu alloys (Φ10 cm × 1 cm, and Φ5 cm × 1 cm) were formed by mixing powders together in a Turbula T2F mixer (Glen Mills Inc, New Jersey, USA) for 30 min, and SLM performed at Key Laboratory of Optoelectronic Materials Chemistry and Physics, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences. The chemical composition of CoCrCu alloy is listed in Table 1. After fabrication, the alloy was processed by solution treatment (1200 ºC, 1 h, fast cooling) and subsequent ageing treatment (900 ºC, 2 h, slow cooling).The same SLM fabricated CoCr based alloys without Cu served as the control group. The SLM fabricated alloys were mechanically polished and further chemically etched (immersion etching) in 36% HCl (in water) for 8h prior to microstructure investigation.

2.2 Elemental distribution and microstructure investigation

Scanning electron microscopy (SEM, 1555 VP-FESEM, Zeiss, Germany) with energy dispersive spectrometer (EDS) was used to determine the elemental distribution within the SLM CoCrCu alloy. Imaging and EDS was performed at an accelerating voltage of 15 kV and working distance of 12 mm. Mapping was performed on a 1024x768 pixel grid, with a dwell time of 1 ms. Metallurgical structures of SLM CoCrCu alloys were observed under an optical microscope (Axiovert 200 MAT). The microstructure analyses of the alloys were performed by X-ray diffraction (XRD) on a Rigaku D/max 2500 pc type X-ray diffraction. The experimental conditions for XRD were CuKa of X ray, tube voltage of 50 kV, tube current of 300 mA, and scan

ACCEPTED MANUSCRIPT

velocity of 1.2 °/min.

2.3 Tensile test

The tensile test was carried out at room temperature on a universal testing machine (Instron, 1251, USA), with a tensile rate of 0.5 mm/min according to the ISO 6892-1: (2009, MOD) using standard specimens (SLM CoCr alloys and SML CoCrCu alloys) of M10×Φ5 mm. Ultimate tensile strength, yield strength and elongation measurements were obtained. An average value of five samples was taken for each material. The fracture morphology of SLM alloys was observed by using optical microscope (Axiovert 200 MAT).

2.4 Potential dynamic polarization

To evaluate the corrosion resistance of the SLM CoCrCu alloys, potential dynamic polarization curves were plotted by Gamry Instruments Reference 600 (USA, Gamry) using a three-electrode system, in which the experimental SLM sample was taken as the working electrode, a platinum foil as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The scan velocity was 0.5 mV/s; test solution was a physical saline (0.9% NaCl solution), and a constant temperature of 37 ºCwas maintained in a water bath.

2.5 Antimicrobial testing

Samples were dipped in 75% ethanol for 20 min and flamed to ensure sterility. Gram-negative bacteria, *Escherichia coli* ATCC25922 (*E.coli*), and Gram-positive bacteria, *Staphylococcus aureus* ATCC25923 *(S.aureus*) used in this study were obtained from Guangdong Microbiology culture center (Guangdong, China). Test species were grown overnight in Tryptone Soya Broth (TSB). The optical density (OD) at 540 nm was adjusted to 0.1 in Phosphate Buffered Saline (PBS). Each SLM sample was placed in a 24-well culture plate. 50 μL of OD adjusted bacterial suspension (population of 1×10^6 /mL) was transferred onto the surface of samples. Samples were then placed in a humidified chamber and incubated at 37 ºC with 10% CO_2 for a period of 24 h. Subsequently each sample was carefully removed and placed in 3 mL of PBS. Approximately 5 glass beads were added to each tube to aid the detachment of the bacteria while spinning the tubes for a period of 30 s at 1500 RPM. Control experiments (culture medium without metal samples) demonstrated such treatment did not adversely affect viability of bacteria. 0.1 mL of the suspension was then removed, plated onto Tryptone Soya Agar (TSA) plates and incubated for 24

ACCEPTED MANUSCRIPT

h. Finally the colonies on the plate were counted to evaluate the antimicrobial ability of SLM samples by means of bacterial survival rate, which is equivalent to the colonies on SLM samples (CoCr or CoCrCu)/colonies on control culture medium [24].

2.6 Live/Dead BacLight bacterial viability staining

Biofilms were studied by using the LIVE/DEAD® BacLightTM Bacterial Viability Kit L7012 (Invitrogen, Molecular Probes, Inc, Eugene, OR, USA) according to the manufacturer's instructions. The kit consists of two stains, propidium iodide (PI) and SYTO-9, which both stain nucleic acids. When used alone, green fluorescent SYTO-9 generally labels all bacteria in a population, whereas red fluorescent PI only penetrates bacteria with damaged membranes, causing a reduction in the green SYTO-9 stain fluorescence. Thus, with an appropriate mixture of the SYTO-9 and PI, bacteria with intact membranes stain fluorescent green, while bacteria with damaged membranes stain fluorescent red. 1 mL of bacterial suspension was dropped onto the tested SLM samples in each well of the 24 well culture plate, which then was incubated at 37 ºC for 24 h. After incubation, the bacteria adhere to the surface of the sample to form a biofilm. Prior to staining, the sample surface was gently washed three times by using 1 mL phosphate buffer saline (PBS) to remove any significant traces of culture medium, planktonic and loosely bound bacteria. 200 µl of the staining solution comprised of 1.5 µl SYTO-9, 1.5 µl PI and 1 mL sterilized distilled water was applied onto the sample for 15 min in the dark at room temperature. After that, the dyed sample was observed using a CLSM (MTC-600, America) at 514/488 nm in argon laser.

2.7 Cytotoxicity assay

The cytotoxicity of the new SLM CoCrCu alloys was determined using the MTT assay. A total of 2×10^4 cells/cm^2 rat marrow mesenchymal stem cells (rMSCs) were seeded on all the SLM samples, and culture medium without metal samples served as control group. The cells were cultured in DMEM culture medium supplemented with 10% (v/v) fetal bovine serum (Biowest, France), antimicrobial agents (100 U/mL of penicillin and 100 µg/mL of streptomycin), and 2 M-glutamine, and incubated at 37 ºC in an atmosphere of 5% CO_2 for 1, 4 and 7 days. The culture medium was changed every 3 days. The MTT solution was prepared by adding thiazolyl blue tetrazolium bromide powder into the PBS, and 10 mL of 5 mg/mL MTT solution was added on the first day. The culture dish was then incubated at 37 ºC under 5% CO_2 and 95% air for 24 h. 100 µL of 10% sodium dodecyl sulphate (SDS, Sigma, USA) in 0.01 M hydrochloric acid was added to each well and re-incubated at 37 ºC in 5% CO_2 and 95% air overnight. Lastly, the absorbance

ACCEPTED MANUSCRIPT

was recorded using a multi-mode detector on the Beckman Coulter DTX 880 at a wavelength of 570 nm with a reference wavelength of 640 nm. The cell viability was determined by the absorbance readings.

2.8 Statistics

All the experiments in this present work were conducted in quintuplicate (n=5) and data were analyzed by one-way ANOVA in SPSS software. The values were expressed as means ± standard deviations and statistical significance was considered when $p < 0.01$.

3. Results:

3.1 Elemental distribution and microstructure analysis

Since the bactericidal properties of CoCrCu alloy would be attributed to the copper ions released from the alloy, the distribution of Cu within the microstructure will directly influence its antibacterial performance. A homogenous microstructure is desirable for this new alloy. Therefore to investigate whether the SLM process can produce a homogenous alloy with a starting point of CoCr and elemental Cu, EDS was performed on a polished section of the alloy. Fig.1a demonstrates the homogenous distribution of Cu (along with all the other elements) while Fig.1b confirms the presence of Cu in the CoCrCu alloy.

The metallography photos of an etched SLM CoCr alloy and SLM CoCrCu sample with three magnifications (50×, 500×, 1000×) are illustrated from optical microscopy (OM) observations in Fig.2. On a macro-level, the accumulative rapid solidification of adjacent melt pools revealed a network appearance of stacked melting pools as reflected in Fig.2b. From magnification images of Fig.2c and 2d, some fine grains were visible in the cellular. The metallography of the SLM CoCrCu alloy is similar to the SLM CoCr alloy, which has been described in Fig.2a and elsewhere [25, 26]. Fig.3 demonstrates the XRD patterns of SLM CoCr and CoCrCu alloys. The two profiles were similar and revealed diffraction graph with peaks at 43.9° (2θ), 50.4° and 74.4°, which indicated that the addition of Cu to this alloy did not change the microstructure of the CoCr based alloy.

ACCEPTED MANUSCRIPT

As shown in Table 2, the tensile and yield strengths of SLM CoCrCu alloy are similar to SLM CoCr alloy. Elongation of the SLM CoCrCu alloys was significantly decreased (p= 0.013) with the addition of Cu, and lower than that of the CoCr alloy. However, the value is still higher than the required elongation value (10%) of related standards (ISO 22674:2006, Dentistry-Metallic materials for fixed and removable restorations and appliances). There was also no difference from the fractographs between SLM CoCrCu and CoCr alloys as demonstrated in Fig.4, meaning that the ductility of SLM CoCrCu was not affected by the addition of Cu. Both fractographs exhibited typical ductile dimple fracture patterns, which further suggests that the addition of Cu in this present work did not affect the mechanical properties of the CoCr based alloys.

3.3 Corrosion resistance

Potential dynamic polarization curves of SLM CoCr and CoCrCu alloys in 0.9% NaCl solution were plotted in Fig.5. Cathodic polarizations of alloys were used in combination with the anodic polarization to determine the corrosion current density (I_{corr}), i.e., corrosion rate, free corrosion potential (E_{corr}) and the pitting corrosion potential (E_{pit}). I_{corr}, E_{corr} and E_{pit} of each experimental sample were listed in Table3. As described in Fig.5, all polarization curves of the experimental samples, either SLM CoCr or SLM CoCrCu alloys are similar in shape and the values of I_{corr}, E_{corr} and E_{pit}, indicating that addition of appropriate amount of Cu in the SLM CoCr based alloys does not affect the corrosion resistance of the alloys.

3.4 Antibacterial studies

Fig.6 highlights a clear inhibitory effect of SLM CoCrCu alloys on bacterial growth after direct contact. The SLM CoCrCu alloys with bacterial suspensions of *E. coli* and *S. aureus*, pathogens associated with implant-related infections and commonly used to preliminary evaluate the antibacterial property of materials, killed 99.99% of each of these species. In contrast, 98% of *E. coli* and 92% of *S. aureus* survived on the surface of SLM CoCr alloys. Consequently, the addition of Cu gives the SLM CoCrCu alloy excellent antimicrobial properties.

3.5 Biofilm inhibition by SLM CoCrCu alloys

Bacterial staining assay was conducted in order to determine the inhibitory effect of SLM CoCrCu alloys on biofilms. Representative CLSM micrographs of both *S. aureus* and *E. coli* after direct contact incubations at 37 ºC for 24 h on surfaces of samples are shown in Fig.7. The images indicate a lower density of bacterial colonization and high proportions of dead bacteria on SLM CoCrCu samples as compared to control samples (Fig.7) for both *S. aureus* and *E. coli*. However,

ACCEPTED MANUSCRIPT

dense biofilms containing high proportions of live bacteria were visible on the SLM CoCr specimens (Fig.7). In addition, multiple microbial aggregations and multilayered biofilms (thickness of about from 18 to 22 μm) were visible on the SLM CoCr samples, however, scattered damaged bacteria without biofilm forming ability were detectable on the surface of SLM CoCrCu alloys as demonstrated from images in the 3D reconstruction (Fig.7). These results indicated that the SLM CoCrCu alloy is able to inhibit the proliferation of bacteria and biofilm formation.

3.6 Cytotoxicity

The results for the cell viability assays (Fig 8) indicated that SLM CoCrCu alloys show no significant differences to SLM CoCr alloys during the test periods of 1d, 4d and 7d, reflecting that the SLM CoCrCu alloys had no cytotoxic effects. Consequently, the addition of Cu offers alloys antibacterial ability but also has satisfactory biocompatibility.

4. Discussion

Within the oral environment, dental materials are preferentially colonized by bacteria with subsequent biofilm formation [27, 28]. Among the bio-active functions proposed for dental materials, antibacterial activity seems the most promising [17, 29]. The use of CoCr based alloys has become popular in recent years as a coping material for use in restorative dentistry [6, 7]. As a widely used coping material, CoCr based alloy is bio-inert which does not possess inherent antibacterial properties, and therefore is easily colonized by bacteria with subsequent development of a biofilm. Although efforts have been devoted to the use of antimicrobial agents treatment, dental implant material associated infection remains an urgent problem that needs to be dealt with.

Elemental Cu is an alloying element often incorporated into metal in order to enhance mechanical properties, wear resistance and corrosion resistance, and copper ions are well known for its antimicrobial properties dating back to the 19th century [20, 21]. Accordingly, appropriated amounts of Cu have been immobilized into the bio-inert metallic biomaterials during the material manufacturing process to provide antibacterial ability as demonstrated in previous studies. For example, Cu has been added into medical grade stainless steel (316L, 304 type stainless steel) and titanium (Ti-6Al-4V) during the material manufacturing process. Results suggested that these Cu-bearing metallic materials kill bacteria and inhibit biofilm formation as shown using *in vitro* and *in vivo* tests. These studies also revealed the significant inhibitory influence of released copper ions from the material upon bacterial survival and biofilm formation. A portion of the released copper ions accumulates at the surface, and a portion diffuses to the surrounding liquid

ACCEPTED MANUSCRIPT

environment [30-35]. Based on the above Cu strategy, the previous study would have immobilized Cu into the cast CoCr alloy during the alloy making process. It was found that this cast CoCrWCuNi alloy possessed obvious antibacterial features against both *E. coli* and *S. aureus*, coupled with biofilm inhibitory effects [36]. According to the above experiment and phase diagram, Cu and CoCr based alloys could melt in each other well under certain high temperature, thus Cu could be immobilized into the CoCr based alloy by using SLM technology.

Based upon these findings, a multi-beneficial SLM CoCrCu alloy was fabricated in the present study. This SLM CoCrCu has three benefits: firstly, it has antibacterial properties and biofilm inhibitory effects which is preferable to that offered via surface modification, which should sustain the antimicrobial activity for longer; secondly, the SLM technology offers the novel chemical design of CoCrCu alloy definitive product density, reduced manufacturing time and costs, minimization of human error, and the prevention of casting defects; thirdly, the removal of Ni element from the chemical composition of the alloy makes it more biocompatible. Thus, the aim of this work was to focus on the antimicrobial ability of this novel dental SLM CoCrCu alloy.

The formation of biofilms on a material usually occurs by two sequential steps. The first is the initial attachment of bacteria to the material surface through van der Waals force using fimbriae or pili. When adhered onto the material, bacteria start to release extra-cellular matrix (ECM), which is beneficial for attachment and also provides protection from the surrounding environmental conditions. The second step is the proliferation and accumulation of bacteria in multi-layers. This proto-biofilm grows through the initial attached bacteria and when the biofilms are large enough, certain areas of the ECM are degraded with enzymes, which lead to dispersal of a portion of the bio-film, allowing cells to disperse and establish more biofilm [37].

When in direct contact with the SLM CoCrCu alloy, a significant number of bacteria are killed,with small percentages of both *E. coli* and *S. aureus* (0.01%) surviving on the surface. This suggests that the alloy has direct bactericidal effects on the early colonizing bacteria and could influence the viability of bacteria present in the early biofilm formation process. Thus, the initial attachment of bacteria to the material's surface is significantly decreased and fewer bacteria are able to produce ECM, which could potentially block biofilm formation. Furthermore, the results of CLSM are consistent with the above analysis and demonstrate that SLM CoCrCu alloy strongly inhibits biofilms *in situ*. In contrast to this, bacterial aggregates and multi-layered biofilms were visible on the surface of SLM CoCr alloys. These current qualitative and quantitative assays

ACCEPTED MANUSCRIPT

suggest that SLM CoCrCu could kill early colonizing bacteria directly and influence the development of the biofilm indirectly. Furthermore, the homogenous distribution of Cu and other elements in the alloy as produced by SLM technology promotes copper ions release, which in turn enhances its antibacterial effect. There are numerous studies focusing on dental materials with anti-biofilm properties that target biofilm prevention. Christian Apel *et al* developed composite materials containing the biomolecule carolacton, which could inhibit the biofilm growth of *Streptococcus mutans* UA159 and is therefore potentially able to prevent secondary caries formation [28]. Jin Feng *et al* recently developed a glass ionomer cement (GIC) containing dimethylaminododecyl methacrylate (DMADDM), which has antibiofilm effects as shown by bacterial morphological changes and decreased biofilm accumulation under oral conditions [17]. Ideally, the antimicrobial activity of dental materials should be addressed as regards to their biofilm inhibitory effects.

The excellent antibacterial effect of SLM CoCrCu is likely to be attributed to the copper ions releasing from its surface. Some studies concerning Cu-bearing biomaterials suggest that their antibacterial effects are due to the copper ions leaching from the material's surface. Wang *et al* revealed that the Cu-rich phases on the surface of the alloy promote the release of copper ions in the cast CoCrWCuNi alloy which could explain its bactericidal activity [36]. Chengtie Wu *et al* fabricated copper-containing mesoporous bioactive glass scaffoldsin which the copper ions instigate antimicrobial activity [38]. A recent study depicted that the formation of voltaic cells caused the release of copper ions from the surface of Cu-bearing stainless steel to the surrounding media, and acting as an antimicrobial agent [39]. Therefore, we hypothesized that the release of copper ions from the surface would contribute to the antibacterial ability for SLM CoCrCu based on the above studies.

Other properties such as mechanical stability, corrosion resistance and biocompatibility are also critical to the novel functional SLM CoCrCu alloy, especially for its future applications in the clinic. As regards to mechanical properties, the strengths of SLM CoCrCu alloy are almost identical to that of SLM CoCr alloy. Although the elongation of SLM CoCrCu alloy was lower than that of CoCr alloy, it meets the requirements of dental materials with no difference depicted from the fractographes for these two alloys. The oral environment is an ideal place for corrosion because of the presence of saliva, acid producing bacterial plaque, changes in pH and temperature related to food or beverage intake, and the action of different medications. Once the corrosion occurs, products released from dental restorations can penetrate the enamel, dentin, and gingiva,

ACCEPTED MANUSCRIPT

and cause local symptoms, such as mucositis or even carcinogenicity and mutagenicity [40-44]. Thus, corrosion behavior is an important consideration for designing dental related biomaterials. The results of potential dynamic polarization curves (Fig.5) and table 3 show that the corrosion resistance of SLM CoCrCu alloy was almost equivalent to SLM CoCr alloys, this meets the requirement of the standard for dental restoration materials (ISO 22674: 2006, Dentistry-Metallic materials for fixed and removable restorations and appliances). Apart from these properties, XRD results indicated that the microstructure of SLM CoCrCu alloy was not altered with the addition of Cu as compared with the SLM CoCr alloy. Thus, immobilizingan appropriate amount of Cu did not change the normal properties of CoCr alloys. More importantly, the biocompatibility of a novel biomaterial should be considered seriously as regards clinical applications, especially for the SLM CoCrCu alloy with improved antibacterial activity. Furthermore, the results for cell viability demonstrated cytocompatibility for the SLM CoCrCu alloy.

Based on the present studies, we conclude that the addition of appropriated amounts of Cu not only offers the SLM CoCrCu alloys excellent bactericidal and antibiofilm properties, but also maintains other beneficial properties of CoCr alloys. Further research as regards to copper ions release, antimicrobial activity and its biocompatibility *in vivo* is necessary in order to provide a more substantial basis for future clinical applications.

Acknowledgements:

This work was financially supported by the National Natural Science Foundation (No. 51171186 and No. 81301329), the National Basic Research Program of China (No. 2012CB619101), Youth Innovation Promotion Association, CAS (No.2014168) and also by UK Royal Academy of Engineering (RAEng Ref. 1213RECI052).

Reference:

[1] I. Yadroitsev, P. Bertrand, I. Smurov, Parametric analysis of the selective laser melting process, Appl. Surf. Sci.253 (2007) 8064-9.

[2] Y.C. Hagedorn, J. Wilkes, W. Meiners, K. Wissenbach, R. Poprawe, Net shaped high performance oxide ceramic parts by selective laser melting, Physics Procedia 5 (2010) 587-94.

[3] N. Xiang, X.Z. Xin, J. Chen, B. Wei, Metalceramic bond strength of Co-Cr alloy fabricatedby selective laser melting. J. Dent.40 (2012) 453-7.

[4] X. Xin, N. Xiang, J. Chen, D. Xu, B. Wei, Corrosion characteristics of a selective laser melted Co-Cr dental alloy under physiological conditions, J. Mater. Sci.47 (2012) 4813-20.

ACCEPTED MANUSCRIPT

[5] X.Z. Xin, J. Chen, N. Xiang, B. Wei, Surface properties and corrosion behavior of Co-Cr alloy fabricated with selective laser melting technique, Cell.Biochem. Biophys.67 (2013) 983-90.

[6] S. Ayyıldız, E.H. Soylu, S. Ide, S. Kılıç, C. Sipahi, B. Pişkin, H.S. Gökçe, Annealing of Co-Cr dental alloy: effects on nanostructure and Rockwell hardness, J. Adv. Prosthodont.5 (2013) 471-8.

[7] Y.S. Al Jabbari, Physico-mechanical properties and prosthodontic applications of Co-Cr dental alloys: a review of the literature, J. Adv. Prosthodont.6 (2014) 138-45.

[8] M. Achtman, M. Wagner, Microbial diversity and the genetic nature of microbial species. Nat. Rev.Microbiol.6 (2008) 431-40.

[9] T.J. Battin, W.T. Sloan, S. Kjelleberg, H. Daims, I.M. Head, T.P. Curtis TP, Microbial landscapes: new paths to biofilm research, Nat. Rev.Microbiol.5 (2007) 76-81.

[10] E. Zaura, B.J. Keijser, S.M. Huse, W. Crielaard, Defining the healthy core microbiome of oral microbial communities. BMC.Microbiol.9 (2009) 259.

[11] A. Lee, H.L. Wang, Biofilm related to dental implants. Implant Dent. 19 (2010)387-393.

[12] N.P.Lang, T. Berglundh, Periimplant diseases: where are we now? -Consensus of the Seventh European Workshop on Periodontology, J. Clin. Periodontol. 38 (2011) 178-181.

[13] H.J.Busscher, M.Rinastiti, W.Siswomihardjo, H.C.van der Mei, Biofilm formation on dental restorative and implant materials. J. Dent. Res. 89 (2010) 657-665.

[14] K. Hojo, S. Nagaoka, T. Ohshima, N. Maeda, Bacterial interactions in dental biofilm development, J. Dent. Res.88 (2009) 982-90.

[15] J. He, Y. Li, Y. Cao, J. Xue, X. Zhou, The oral microbiome diversity and its relation to human diseases, Folia.Microbiol (Praha) 60 (2015) 69-80.

[16] Z.J. Wang, Y. Shen, M. Haapasalo, Dental materials with antibiofilm properties, Dent. Mater.30 (2014) e1–e16.

[17] J. Feng, L. Cheng, X.D. Zhou, Hockin H.K. Xu, Michael D. Weir, M. Meyer, H. Maurer, Q. Li, M. Hannig, S. Rup, In situ antibiofilm effect of glass-ionomer cement containing dimethylaminododecyl methacrylate., Dent. Mater. (2015) Article in press.

[18] Y. Takahashi, S. Imazato, A.V. Kaneshiro, S. Ebisu, J.E. Frencken, F.R. Tay, Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach, Dent. Mater. 22 (2006) 647-52.

[19] K. Zhang, L. Cheng, S. Imazato, J.M. Antonucci, N.J. Lin, S. Lin-Gibson, Effects of dual antibacterial agents MDPB and nano-silver in primer on microcosm biofilm, cytotoxicity and dentine bond properties, J. Dent. 41(2013) 464-74.

[20] G.A.A. Ferns, D.J. Lamb, A. Taylor, The possible role of copper ions in atherogenesis: The blue janus. Atherosclerosis 133 (1997) 139-152.

ACCEPTED MANUSCRIPT

[21] C.G. Fraga, Relevance, essentiality and toxicity of trace elements in human health, Mol. Aspects. Med. 26 (2005) 235-244.

[22] J.L. González-Carrasco, G. Ciapetti, M.A. Montealegre, Evaluation of mechanical properties and biological response of an alumina-forming Ni-free ferritic alloy. Biomaterials, 26 (2005) 3861-3871.

[23] G. Ciapetti , J.L. González-Carrasco, L. Savarino, Quantitative assessment of the response of osteoblast and macrophage-like cells to particles of Ni-free Fe-base alloys. Biomaterials 26 (2005) 849-859.

[24] K. Memarzadeh, A.S. Sharili, H. Jie H, S.C.F. Rawlinson, R.P. Allaker, Nanoparticulate zinc oxide as a coating material for orthopedic and dental implants. Journal of Biomedical Materials Research Part A, 103(2014):981-989.

[25] E. Bettini, T. Eriksson, M. Boström, C. Leygraf, J. Pan, Influence of metal carbides on dissolution behavior of biomedical CoCrMo alloy: SEM, TEM and AFM studies, Electrochim.Acta.56 (2011) 9413-20.

[26] E. Bettini, C. Leygraf, C. Lin, P. Liu, J. Pan, Influence of grain boundaries on dissolution behavior of a biomedical CoCrMo alloy: in-situ electrochemical-optical, AFM and SEM/TEM studies, J.Electrochem. Soc.159 (2012) C422-7.

[27] S. Imazato, Antibacterial properties of resin composites and dentin bonding systems, Dent. Mater. 19 (2003) 449-57.

[28] A. Christian, B. Andree, R. Anke, C. Georg, W.D. Irene, Dental composite materials containing carolacton inhibit biofilm growth of Streptococcus mutans, Dent. Mater. 29 (2013) 1188-1199.

[29] Z. Wang, Y. Shen, M. Haapasalo, Dental materials with antibiofilm properties, Dent. Mater. 30 (2014) e1–16.

[30] L. Ren, K. Yang, L. Guo, H.W. Chai, Preliminary study of anti-infective function of a Cu-bearing stainless steel,Mat. Sci. Eng. C-Mater, 32 (2012) 1204-1209.

[31] L. Ren, K. Yang, Bio-functional Design for Metal Implants, a New Concept for Development of Metallic Biomaterials, J. Mater. Sci. 20 (2013) 1005-1110.

[32] L. Ren, Z. Ma, M. Li, Y. Zhang, W.Q. Liu, Z.H. Liao, K. Yang, Antibacterial Properties of Ti-6Al-4V-xCu Alloys, J. Mater. Sci.30 (2014) 699-705.

[33] D. Zhang, L. Ren, Y. Zhang, N. Xue, K. Yang, M. Zhong, Antibacterial activity against *Porphyromonasgingivalis* and biological characteristics of antibacterial stainless steel, Colloid. Surface. B. 105 (2013) 51-57.

[34] L. Ren, L. Nan, K. Yang, Study of copper precipitation behavior in a Cu-bearing austenitic antibacterial stainless steel,Mater. Design. 32 (2011) 2374-2379.

[35] L. Ren, J.M. Zhu, L. Nan, K. Yang, Differential scanning calorimetry analysis on Cu precipitation in a high Cu austenitic stainless steel, Mater. Design. 32 (2011) 3980-3985.

[36] S. Wang, C.G. Yang, L. Ren, M.G. Shen, K. Yang, Study on antibacterial performance of Cu-bearing cobalt-based alloy, Mate. Lett. 129 (2014) 88-90.

ACCEPTED MANUSCRIPT

[37] R.A. Carla, C. Davide, S. Pietro, M. Lucio, W.C. John, Biofilm formation in *Staphylococcus* implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials, Biomaterials 33 (2012) 5967-82.

[38] C. Wu, Y. Zhou, M. Xu, P. Han, L. Chen, J. Chang, Y. Xiao, Copper- containing mesoporous bioactive glass scaffolds with multifuntional properties of angiogenesis capacity, osteostimulation and antibacterial activity, Biomaterials 34 (2013) 422-433.

[39] H.W. Chai, L. Guo, X.T. Wang, Y.P. Fu, J.L. Guan, L.L. Tan, L. Ren, K. Yang, Antibacterial effect of 317L stainless steel contained copper in prevention of implant-related infection in vitro and in vivo, J. Mater. Sci: Mater. M. 22 (2011) 2525-2535.

[40] M. Kuhta, D. Palvin, M. Slaj, S. Varga, M.L. Varga, M. Slaj, Type of archwire and level of acidity: effects on the release of metal ions from orthodontic appliances, Angle Orthod.79 (2009) 102-10.

[41] Y. Okazaki, E. Gotoh, Metal release from stainless steel, Co-Cr-Mo-Ni-Fe alloys in vascular implants, Corros. Sci. 50(2008) 3429-38.

[42] N. Samet, B. Resheff, S. Gelbard, N. Stern, A CAD/CAM system for the production of metal copings for porcelain-fused-to-metal restorations, J.Prosthet Dent.73 (1995) 457-63.

[43] A.I. Kovalev, D.L. Vainshtein, V.P. Mishina, V.I. Titov, V.F. Moiseev, N.K. Tolochko, Selective laser sintering of steel powders to obtain products based on SAPR- models, Metallurgist 44 (2000) 206-9.

[44] E.I. McGinley, A.H. Dowling, G.P. Moran, G.J. Fleming, Influence of *Streptococcusmutans* on base-metal dental casting alloy toxicity, J. Dent. Res.92 (2013) 92-7.

ACCEPTED MANUSCRIPT

Fig.1 EDS maps of metal elements confirming the uniform distribution of Cu and all other elements in the alloy (a). Full spectra from the SLM CoCrCu alloy showing the presence of Cu in the alloy (b)

Fig.2 Metallography electron micrographs of an etched SLM CoCr alloy (a). SLM CoCrCu sample at three different magnifications, (b) 50×, (c) 500×, (d) 1000×, Pattern-like network appearance of stacked melting pools on the macro-level and some fine grains were visible in the cellular.

Fig.3 X-ray diffraction patterns of SLM CoCr and CoCrCu alloys with characteristic peaks at 43.9° (2θ), 50.4° and 74.4°

Fig.4 Fractographs after tensile testing of SLM CoCr (a) and CoCrCu (b) alloys. There was no difference from the fractographs between SLM CoCrCu and CoCr alloys, indicating that the ductility of SLM CoCrCu was not affected by the addition of Cu

Fig.5 Potential dynamic polarization curves of SLM CoCr and CoCrCu alloys in 0.9% NaCl solution. Both SLM CoCr and SLM CoCrCu alloys were similar in shape indicating similar corrosion resistance properties

Fig.6 Bacteria survival rates of *E. coli* (a) and *S.aureus* (b) after direct contact with different materials (Control, CoCr and CoCrCu) for 24 h at 37 ºC to evaluate the antimicrobial properties. Control was culture medium without metal samples. ** Indicate significant differences (p<0.01) compared with control and CoCr groups

Fig.7 CLSM images (including plane and 3D reconstruction graphs) of *E. coli* (a) and *S.aureus* (b) after direct contact with SLM CoCr and CoCrCu alloys for 24 h at 37 ºC to evaluate the antibiofilm property

Fig.8 OD values of rat marrow mesenchymal stem cells (rMSCs) for different groups (Control, CoCr and CoCrCu) at different time points by using MTT assay at a wavelength of 570 nm with a reference wavelength of 640 nm. Control represents culture medium (DMEM) without metal samples. ** Indicate significant differences (p<0.01) compared with CoCr and CoCrCu groups at each time point

ACCEPTED MANUSCRIPT

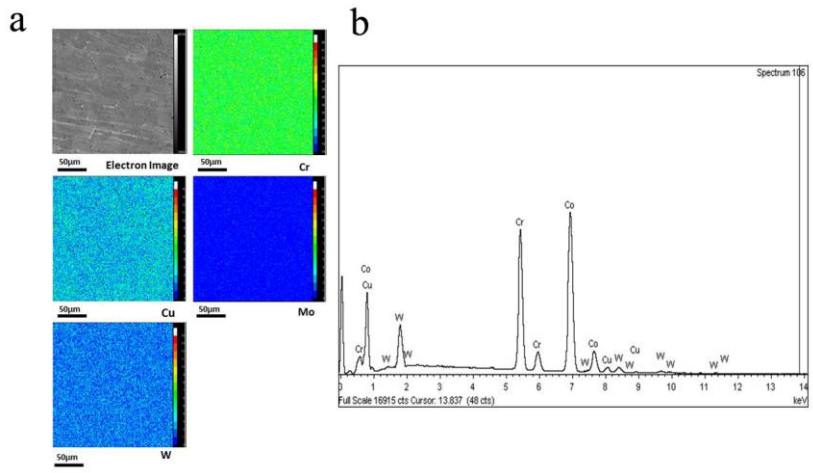


Figure 1

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

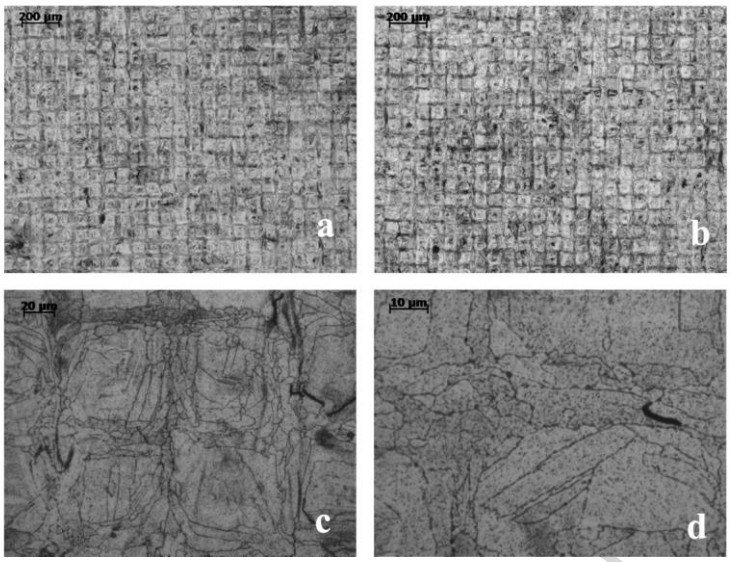


Figure 2

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

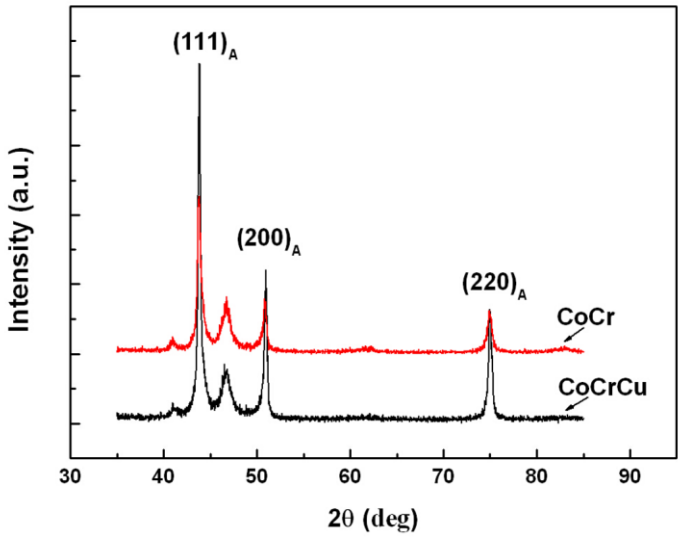


Figure 3

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

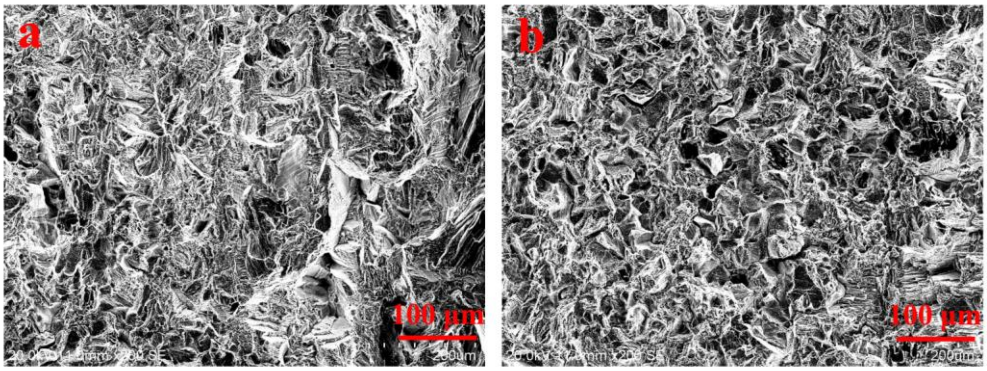


Figure 4

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

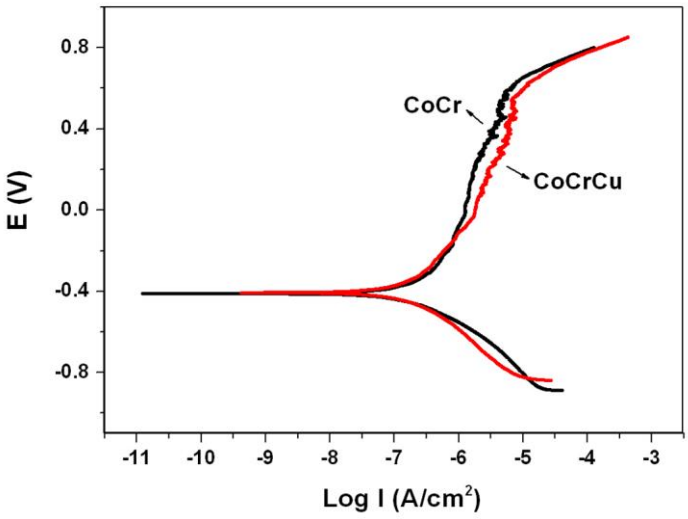


Figure 5

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

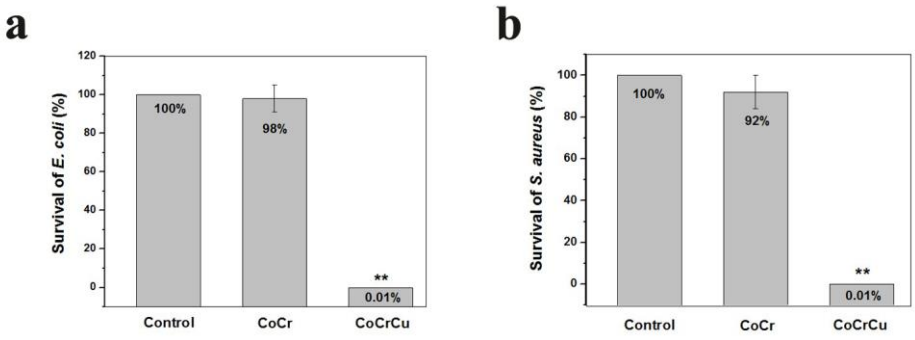


Figure 6

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

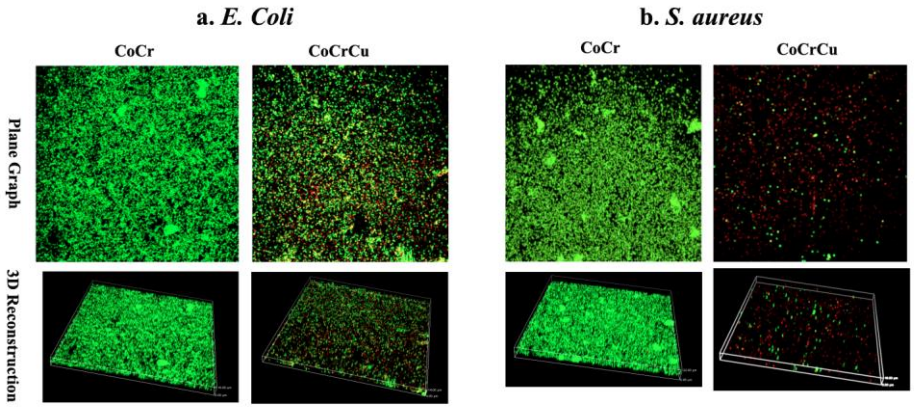


Figure 7

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

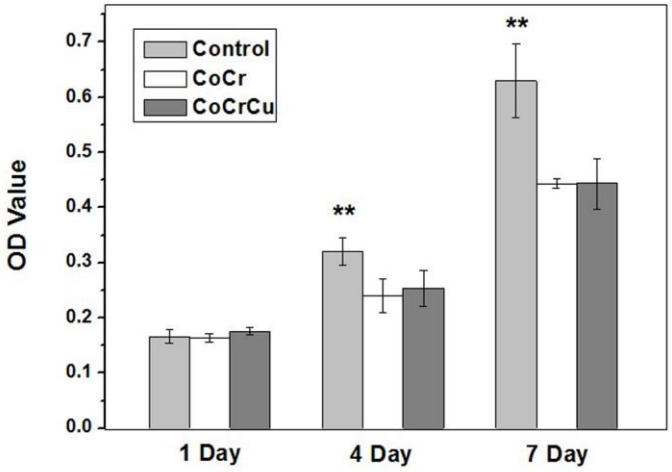


Figure 8

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table-1. Chemical composition (wt.%) of SLM CoCrCu alloy

Co	Cr	Cu	W	Si	Fe	Ni	Mn	Cd	Be	Nb
58.2	28.21	2.8	8.98	1.49	0.82	<0.1	<0.3	<0.0001	<0.01	<0.05

Table 2 - Mean (standard deviation) of mechanical properties of SLM CoCr and CoCrCu alloys

Virable	Yield Strength (MPa)	Tensile Strength (MPa)	Elongation (%)
SLM CoCr alloy	565±5.68	1084±12.56	26.5±1.2
SLM CoCrCu alloy	636.5±8.12**	991±13.98	13.5±0.45**

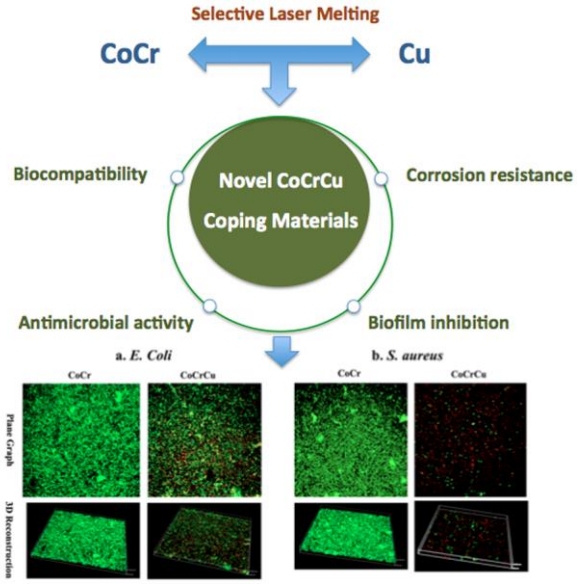
** Indicates significant differences (p<0.01) between groups

Table 3 Mean and standard deviations for kinetic parameters associated with corrosion process of SLM CoCr and CoCrCu alloys in 0.9% NaCl solution

Virable	E_{corr},mV	I_{corr},nA	E_{pit},mV
SLM CoCr alloy	-411.0±22.15	211.8±10.0	582.8±25.0
SLM CoCrCu alloy	-406.2±16.73	217.2±9.45	562.1±22.34

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



Graphical abstract

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

1. Novel CoCrCu alloys were fabricated by using selective laser melting (SLM).
2. SLM CoCrCu alloys showed satisfied antimicrobial and antibiofilm activities.
3. SLM CoCrCu alloys have no cytotoxic effect on normal cells.
4. Other properties of SLM CoCrCu alloys were similar to SLM CoCr alloys.
5. SLM CoCrCu alloys have the potential to be used as coping metals.

ACCEPTED MANUSCRIPT